Thesis/
Reports
Johnson,
L. A.

GENETICS AND INTROGRESSION OF WILDLAND FORB POPULATIONS AND SEEDINGS

01-JV-11222073-166

FINAL REPORT

SUBMITTED TO:

DURANT MACARTHUR
U.S. FOREST SERVICE
ROCKY MOUNTAIN RESEARCH STATION

REGARDING:

GENETICS AND INTROGRESSION OF WILDLAND FORB POPULATIONS AND SEEDINGS.

A JOINT RESEARCH / COOPERATIVE AGREEMENT WITH THE U.S. FOREST SERVICE
ROCKY MTN. RESEARCH STATION – SHRUB SCIENCES LABORATORY
AND BRIGHAM YOUNG UNIVESITY

PREPARED BY:

LEIGH A. JOHNSON
ASSOCIATE PROFESSOR
DEPARTMENT OF INTEGRATIVE BIOLOGY
BRIGHAM YOUNG UNIVERSITY, PROVO UT 84602

TAINA M. PRICE
M.S. CANDIDATE
DEPARTMENT OF INTEGRATIVE BIOLOGY
BRIGHAM YOUNG UNIVERSITY, PROVO UT 84602

LOREEN A. WOOLSTENHULME
ASSOCIATE PROFESSOR
DEPARTMENT OF INTEGRATIVE BIOLOGY
BRIGHAM YOUNG UNIVERSITY, PROVO UT 84602

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PROJECT SUMMARY

In cooperation with personnel at the Rocky Mountain Research Station Shrub Sciences Laboratory, four candidate forbs were selected for genetic study in 2001. These species are: *Linum perenne*, blue flax (Linaceae); *Sphaeralcea grossularifolia*, gooseberry-leaf globemallow (Malvaceae); *Penstemon palmeri*, Palmer's penstemon (Scrophulariaceae), and *Hedysarum boreale*, Northern Sweetvetch (Fabaceae).

We focused on the genetic diversity of wildland forb populations, both natural and reseeded, to assess patterns of genetic variation and assess whether introgression was observable between native and reseeded populations. Our report summarizes our findings for the first three of these four species, with the fourth species eliminated due to difficulty in locating extant reseeded populations.

Our findings, and implications of our findings, vary by species. In general, however, we observed that for the study species, little consideration was given to their presence at a site prior to disturbance and revegetation seeding. Furthermore, two of the three species are often mis-identified or at least mischaracterized as to species (e.g., sites said to have been reseeded with *S. grossularifolia* are more commonly reseeded with *S. munroana*).

Our analyses support the designation of *Linum perrene* and *Linum lewisii* as distinct species, probably incapable of hybridization. Species specific DNA markers were found for both species, as well as for the several species of *Penstemon* found growing with reseeded populations of *Penstemon palmeri*. Genetic diversity appears greatest in *Sphaeralcea* species, to the extent that assessing homology across individuals for all ISSR bands could not be accomplished with confidence as to accuracy.

Presentations of this work were made to Society for Range Mangament meetings in February of 2004 [, and the American Botanical Society meetings in August 2004. A master's thesis (Taina M. Price) is in preparation reporting the work for Linum Lewisii, with a manuscript anticipated for publication to follow by December 2006.

Matheson, T., L. A. Johnson, D. MacArthur, D. Johnson, and S. McMurray. Assessing genetic variation within and between in natural and reseeded populations of two native forbs. Society for Range Mangament meetings, February 2004.

Matheson, T., Johnson, D. P., McMurry, S. L., Johnson, L. A. Using ISSRs to assess genetic variation within and between populations of blue flax. American Botanical Society meetings, August 2004.

INTRODUCTION

Vegetation reseeding following fires or other land disturbances is often conducted to facilitate re-establishment of biological communities and prevent further devastation to the abiotic components of disturbed lands. While attempts are made to reseed areas with native plants of known origin, little concern has been placed on genetic diversity in reseeded populations, whether populations sampled are genetically and ecologically exchangeable with the populations being replaced, whether the species introduced through reseeding are, in fact, conspecific with native relatives that inhabited reseeded sites prior to disturbance, or even if the reseeded species or their relatives existed at such sites prior to reseeding efforts. Reseeding with species or locally-adapted populations that are significantly different in their ecological requirements than the native populations or species that existed prior to disturbance can have negative consequences for long-term establishment of reseeded species, as well as impacts on the adjacent native flora via hybridization and introgression between native and reseeded populations.

In 2001, we initiated a cooperative agreement to investigate genetic variation in reseeded populations and the extent of gene flow between such populations and native stands in adjacent areas. In cooperation with personnel at the Rocky Mountain Research Station – Shrub Sciences Laboratory, four candidate species were selected for study: *Linum perenne*, blue flax (Linaceae); *Sphaeralcea grossularifolia*, gooseberry-leaf globemallow (Malvaceae); *Penstemon palmeri*, Palmer's penstemon (Scrophulariaceae), and *Hedysarum boreale*, Northern Sweetvetch (Fabaceae).

This final report details our investigation of genetic variation and geneflow in wildland forb populations and seedings of the species listed above in Utah.

Project Objectives

The purpose of this agreement was to explore the populations genetics of forb species used in revegetation plantings with an emphasis on the possible introgression into indigenous populations or congeners of seeded species to help managers make informed decisions in choosing revegetation planting stock.

1. Identify populations for each target species that have been used in revegetation plantings as source populations, populations seeded as revegetation plantings, and adjacent indigenous "populations" considered conspecific or congeneric into which introgression could be assessed.

2. Collect material (leaves or seeds) from up to 50 individuals per identified population for genetic analysis.

3. Isolate DNA for DNA-based analyses, and leaf proteins for isozyme analyses.

4. Analyze data

5. Prepare and submit a final report [Original target: September 2004; extension target: 20 June 2006]

Caveats

Two confounding factors caused us to alter our original experimental design – all alterations were made in consultation with our cooperator at the Rocky Mountain Research Station – Shrub Sciences Laboratory. First, the ideal experimental design originally conceived for sampling populations proved difficult to find in nature. Known reseeded sites using the target species were limited (for some species more that others); of the reseeded sites identified, relocating the source populations proved difficult or impossible; and located reseeded sites did not always have adjacent indigenous populations of the same species or congeners. Second, both 2001 and 2002 were extreme drought years in Utah. Based on contacts and cooperation from numerous Forest Service and Bureau of Land Management biologists, an extensive site search was made in 2002 of possible populations by Braken Davis, a temporary Forest Service employee, either by himself or with Leigh Johnson. Plant presence was documented during these site visits, but tissue collection was impossible. Consequently, the time frame of this study was extended to facilitate successful completion of surveys for genetic variation.

Target Species

***Linum perenne* L.**

***Linum lewisii* Pursh.**

Blue flax has very showy flowers, and several cultivars have been developed (Meyer & Kitchen 1994). The cultivar that is widely used in both ornamental and rehabilitation plantings was developed as a cooperative effort of the Soil Conservation Service, the USDA Forest Service Shrub Sciences Laboratory, and the Forest Service Plant Materials Center in Aberdeen, Idaho (Meyer & Kitchen 1994, USDA 1980). This cultivar, 'Appar' was selected for outstanding beauty, vigor, and competitiveness with understory grasses (USDA 1980). Appar was assigned to *L. lewisii* when it was released in 1980 (USDA 1980, Ogle 2002). However, Appar is heterostylous, as is the European *L. perenne* (Pendleton et al., 1993*a*, 1993*b*), whereas the native *L. lewisii* is homostylous. Thus, despite its fine attributes, the widely planted Appar is a form of *L. perenne*, distinct from *L. lewisii*. We assessed genetic variation in both the native L. lewisii and the introduced L. perenne cv. Appar, including several sympatric populations of these species.

***Penstemon palmeri* A. Gray**

***Penstemon eatonii* A. Gray**

***Penstemon cyananthus* Hook var *sublaber* (A. Gray) N. Holmgren**

***Penstemon cyanocaulis* Payson**

Palmers penstemon is a short lived perennial commonly included in road side reseeding mixes due to its tall stature and showy light pink flowers. *Penstemon palmeri* var. *eglandulosus* (Keck) N. Holmgren is native to southern Utah, but the variety commonly included in reseeding mixtures because of its commercial availability, *P. palmeri* var. *palmeri*, is not (Cronquist et al. 1984. The potential for hybridization between varieties has been used to limit reseeding of var. palmeri near the narrowly endemic *P. palmeri* var. *macranthus* in Nevada (http://www.nv.blm.gov/bmountain/eis/Spenser_Hot_Springs/6_Responses%20to%20Public%20Comments.pdf), and is a concern for native populations of *P. bicolor* as well (Glenne and Tepedino 2000). We studied three populations of seeded Palmer's penstemon sympatric with other penstemon species.

***Sphaeralcea grossulariifolia* (Hook. & Arn.) Rydberg**
***Sphaeralcea munroana* (Dougl.) Spach**
***Sphaeralcea ambigua* A. Gray**

Sphaeralcea grossularifolia is often listed as a component of seeding mixes. Our site visits suggest that in several instances, the species contained in the reseeding mixes is actually *S. munroana*. *Sphaeralcea* is a difficult genus taxonomically with species that commonly intergrade in sympatric zones. Such hybridization is facilitated by weak sterility barriers between species and obligate-outcrossing reproduction facilitated by insect vistors and self-incompatiblity. Indeed, hybrids and introgressents with intermediate morphologies were common at several sites. Our sampling thus includes mixed populations, populations of *S. grossularifolia*, and at one location, *S. ambigua* and putative hybrids between introduced *S. grossularifolia* and this species.

***Hedysarum boreale* Nutt.**

Hedysarum boreale is likely to see increasing use in seeding mixtures given that funding for reseeding may include restrictions on forb use unless there is demonstrated value to the establishment of other species or other benefit to the environment. As a nitrogen fixer, *Hedysarum* can add such value. Nevertheless, after identifying only two seeded populations of Hedysarum, and finding no plants in 2002 or 2003, further work with this species was terminated.

Overview of Genetic Screening Methodology

DNA-based Methods. Genetic variation was assayed using DNA-based markers under the supervision of Dr. Leigh Johnson. Inter-simple-sequence-repeats (ISSRs) were employed to eliminate the use of radiation, reduce many of the problems associated with repeatability using RAPDs, and because such markers have shown great utility in assessing variation within and between populations and species, particularly with identifying species-specific bands that can be used to track introgression from one species to another (Wolfe et al. 1998).

ISSRs are hypervariable markers based on single-primer PCR reactions in which the primers are derived from di- and trinucleotide repeats. The primers incorporate anchoring sequences that are generally random and may contain redundant bases. Unique banding profiles are produced by different anchoring sequences with a common microsatellite motif (Wolfe et al., 1998). ISSRs are dominant, anonymous markers similar to RAPDs; they are scored as binary characters (band presence or absence). ISSRs were initially used chiefly in crop studies (e.g., Liu & Wendel 2001), but in recent years they have been applied to studies of genetic variation, gene flow, and hybridization in native plant species (e.g., Wolfe et al. 1998, Esselman et al. 1999, Martin & Sanchez-Yelamo 2000, Camacho & Liston 2001, Culley & Wolfe 2001, Kimball et al. 2001, Wolfe & Randle 2001, McGlaughlin et al. 2002, Smith & Bateman 2002, Archibald et al. 2004, Alexander et al. 2004 etc.) and naturalized weeds (e.g., Meekins et al. 2001, Tranel & Wassom 2001, Benharrat et al. 2002). ISSRs can produce large numbers of variable loci even for species in which little sequence data is available.

ISSRs have been successfully used to assess genetic diversity and relationships and to generate genetic fingerprints for identification of germplasm in cultivated flax, *Linum usitatissimum* L. (Wiesner & Wiesnerova 2003, Wiesnerova & Wiesner 2004), a close relative of the *L. perenne* group. They have also been employed to assess species boundaries and introgression in Penstemon (Wolfe et al. 1998).

Protein-based Methods. Genetic assays based on protein electrophoresis (isozyme analyses) were initiated under the supervision of Dr. Loreen Woolstenhulme. Very little useful data was obtained, with degradation of tissue samples complicating assays from the material collected originally. Such work is not further elaborated in this report.

MATERIALS AND METHODS

Taxon Sampling

Drought in the intermountain west in 2001 and 2002 confounded original plans for species sampling and experimental design. Loreen Woolstenhulme and Leigh Johnson made a presentation at a western regional Forest Service meeting in Park City, Utah in April 2001 to solicit help in locating reseeded populations. In 2002, Braken Davis, a temporary employee of the Rocky Mountain Shrub Lab followed up on all leads, pursued additional contacts through BLM offices, and made site visits to many potential sites. Leigh Johnson accompanied Bracken on several site visits in June/July 2001. While species presence could be identified from plant remains for globe mallow, palmer's penstemon, and blue flax at several sites, sampling of useful material for laboratory analysis was impossible. *Hedysarum*, for which only two seeded sites could be located without any evidence of the species presence, was eliminated from further consideration. Consequently, plant samples were first collected in 2003.

Linum. Native *L. lewisii* and seeded *L. perenne* were collected from sites in north-central Utah. A total of seven *L. lewisii* populations and five *L. perenne* populations were sampled (Table 1). *L. perenne* sites were identified from seeding records provided by the USDA Forest Service and other agencies, and by personal observation. One site had been reseeded following wildfire. The other four were roadside rehabilitation seedings. Native populations of *L. lewisii* were identified using herbarium collection data, personal observations, and previous field work. Stan Kitchen of the Shrub Sciences Laboratory provided a list of collection sites in the Intermountain Region for native *L. lewisii*. These sites were among twenty-one previously used in comparitive studies by Shrub Lab researchers (e.g., Meyer & Kitchen 1994).

One *L. lewisii* and one *L. perenne* population (at Holden; see Table 1) were sympatric. The site had been burned over, and 'Appar' was included in the revegation seed mixture, while naturally occurring *L. lewisii* persisted, presumably in patches of lesser fire intensity and by resprouting from its seed bank. One *L. lewisii* population (Ibapah) was thought to be potentially distinct, due to its unusual habitat and soil type, and subtly different appearance (short stature, grey color). Another population (Maple Grove) was the source of a new germplasm release of *L. lewisii* by the Plant Materials Center. Selected for its outstanding beauty, vigor, and competitiveness, the Maple Grove germplasm was released in 2003 by the same consortium that released *L. perenne* 'Appar' in 1980 (Ogle et al. 2003).

We sampled approximately 48 individuals from each population (Table 1). Sampled individuals were selected arbitrarily, avoiding close neighbors as these may be close genetic relatives. We made an effort to cover the observed extent of the population. In some cases (e.g., LIPE2, LILEA, and LILEB) the population was sampled exhaustively (i.e., all or almost all individuals sighted were sampled) without reaching the target 48 individuals. Voucher specimens from each site were collected and pressed.

Though *L. lewisii* and *L. perenne* 'Appar' have a nearly identical growth habit and general appearance, and are very difficult to distinguish on herbarium sheets, we were able to readily distinguish the two species in the field. At the Holden site, where both species grew sympatrically, we could confidently assign each individual to one or the other species based on flower color, vegetative color, and the presence or absence of heterostyly. We observed no apparent morphological intermediates.

Table 1. Blue flax (*Linum* spp.) populations

Population	Site	Native/ seeded	Latitude / Longitude	# sampled
LILE1	Eureka	Native	N 39° 57.74' W 112° 08.56'	48
LILE2	Holden	Native	N 39° 03.25' W 112° 14.37'	47
LILE3	Cove Fort	Native	N 38° 36.59' W 112° 36.87	48
LILE4	Maple Grove	Native	N 39° 01.29' W 112° 04.59'	48
LILE5	Ibapah	Native	N 40° 2.50' W 114° 2.00'	48
LILEA	Pony Express	Native	N 40° 06.12' W 113° 54.57'	9
LILEB	Richmond	Native	N 41° 55.95' W 111° 45.99'	6
LIPE1	Eagle Mountain	Seeded	N 40° 23.02' W 111° 57.41'	48
LIPE2	Holden	Seeded	N 39° 03.29' W 112° 14.42'	30
LIPE3	Salt Creek Canyon	Seeded	N 39° 43.05' W 111° 44.56'	48
LIPE4	Ephraim Canyon	Seeded	N 39° 20.17' W 111° 32.45'	48
LIPE5	Provo Canyon	Seeded	N 40° 19.73' W 111° 37.42'	48

LILE = *Linum lewisii*; LIPE=*Linum perenne*.

Penstemon. Native and seeded Penstemon were collected from sites in north-central to southern Utah. A total of seven populations were sampled, with population sizes ranging from one to 47 individuals. With the exception of this 47-individual sample of *Pentemon palmeri*, all individuals that could be reasonably located were sampled; thus, the number of individuals from populations of this genus is lower than the number sampled for the other genera in this study. Palmer's penstemon was represented by four seeded populations. Native penstemons of other species sympatric with these seeded populations were also sampled at three of the locations (Table 2). In all cases, discernable morphological intergradation between species was non-existent, and samples were thus assigned to species in the field.

Table 2. Penstemon (*Penstemon* spp.) populations

Population	Site	Native/ seeded	Latitude / Longitude	# sampled
PEPA A	Twist Hollow	Seeded	N 37° 9.42' W 113° 36.12'	10
PEEA B	Twist Hollow	Native	N 37° 9.42' W 113° 36.12'	1
PECC C	Hwy 70	Native	N 38° 35.21' W 112° 29.42'	15
PEPA D	Hwy 70	Seeded	N 38° 35.21' W 112° 29.42'	16
PECA E	Pole Canyon	Native	N 39° 46.68' W 111° 41.91'	28
PEPA F	Pole Canyon	Seeded	N 39° 46.68' W 111° 41.91'	47
PEPA G	Ephraim Canyon	Seeded	N 39° 20.17' W 111° 32.45'	15

PEPA = *P. palmeri*; PEEA = *P. eatonii*; PECC = *Penstemon cyanocaulis*; PECA = *P. cyananthus* var *sublaber*.

Sphaeralcea. Native and seeded *Sphaeralcea* were collected from sites in north-central to southern Utah. A total of eight populations were sampled (Table 3), Of these, three were seeded populations and four are putatively native populations. Two populations, SPAM1 and SPGR1 are sympatric, with high confidence in the identify of the species collected at these sites. Three individuals included in SPGR1 were marked as putative hybrids between *S. ambigua* and *S. grossularifolia* at the time of collection. Four of the SPGR populations (Table 2) were noted as hybrid swarms at the time of collection. We observed, as suggested by a colleague, that *S. munroana* is often used in reseeding mixes although reseeding records specify *S. grossularifolia.* SPGR3, the Tebbs Pond population, for example, had both putatively pure and hybrid forms of *S. munroana* on the reseeded portion of the range, although only *S. grossularifolia* was located in adjacent, native stands (SPGR4). With apparent introgressent forms noted at these sites, an effort was made to collect material, with notation, across the range of morphological variation observed. However, no attempt was made to collect *S. grossularifolia* versus *S. munroana* separately because apparent hybrids spanned the full range of intermediacy between the parental types such that "pure" species versus 90% introgressants could only be defined by subjective and arbitrary means.

Table 3. Globe mallow (*Sphaeralcea* spp.) populations

Population	Site	Native/ seeded	Latitude / Longitude	# sampled
SPAM1	Twist Hollow	Native	N 37° 9.42' W 113° 36.12'	53
SPGR1	Twist Hollow	Seeded	N 37° 9.42' W 113° 36.12'	22+3
SPGR2*	Goshen Canyon	Native	N 39° 53.96' W 111° 53.36'	47
SPGR3*	Tebbs Pond	Seeded	N 38° 03.13' W 112° 58.72'	48
SPGR4	Minersville	Native	N 38° 10.58' W 113° 3.26'	48
SPGR5*	Willow Creek	Native	N 39° 01.16' W 111° 46.30'	48
SPGR6*	West Mountain	Seeded	N 40° 2.5' W 111° 48.0'	48
SPGR7	Cove Fort	Native	N 38° 36.59' W 112° 36.83'	48

SPAM = *S. ambigua*; SPGR = *Sphaeralcea grossularifolia*; * indicates probably mixed populations, in most cases with *S. munroana*, but in some cases possibly with *S. coccinea.*

Molecular Methods

DNA extraction. Leaf material from each sampled individual was preserved by desiccation in silica gel immediately upon collecting in the field. We extracted total genomic DNA using a modified CTAB microprep protocol (Doyle & Doyle 1987, Cullings 1992). Approximately 115 mg of silica-dried leaves were ground in a warm 2X CTAB buffer containing 2% w/v PVP and 0.5% β-mercaptoethanol. 5 μL of RNAse A (10 mg/mL) was added to each, and the samples were incubated at 65° C for four hours. DNA was then extracted with chloroform and precipitated overnight with cold 7.5 M ammonium acetate and isopropanol. Pellets were washed with 70% ethanol, dried and resuspended in 70 μL 1X TE buffer. We made 1:10 dilutions in deionized H_2O of each extract for use as template DNA in ISSR-PCR.

ISSR-PCR. Considerable effort was expended to optimize ISSR analyses in our lab, using work by others (e.g., Wolfe et al. 1998; Blair et al. 1999; Liu and Wendel 2001; Wiesner and Wiesnerová 2003) as a guide. Experimentation focused on clarifying banding patterns and ensuring repeatability of results. For example, a two-step ISSR method published based on work

in flax (Wiesner and Wiesnerová 2003) gave disturbingly different patterns from the same individuals in our early assays. The methods we report below gave reproducible results that could typically be scored with high confidence.

After screening primers used in previous ISSR studies of native plant species, we designed our own set of primers based on common plant nuclear SSR sequences. We screened twelve primers consisting of nine repeats each of a dinucelotide motif, followed by a single 3' anchoring base, and six primers consisting of seven repeats each of a trinucleotide repeat, followed by a single 3' anchoring base. These primers were $(GA)_9A$, $(GA)_9T$, $(GA)_9C$, $(GT)_9A$, $(GT)_9T$, $(GT)_9C$, $(CA)_9T$, $(CA)_9G$, $(CA)_9A$, $(CT)_9A$, $(CT)_9T$, $(CT)_9G$, $(CAG)_7G$, $(CAG)_7A$, $(CAA)_7T$, $(CAA)_7G$, $(CTC)_7G$, and $(CTC)_7A$. After screening all primers with a few individuals from each population of our target species, we selected several primers to amplify in all individuals of each of the three forbs. The selected primers produced the clearest banding patterns, with a large number of variable bands across the individuals used for screening, and appeared to produce reliably repeatable banding patterns across repeated sets of reactions. For *Linum*, we selected six dinucleotide and two trinucleotide primers: $(GA)_9A$, $(GA)_9T$, $(GA)_9C$, $(CT)_9G$, $(CA)_9G$, $(CA)_9A$, $(CAA)_7T$, and $(CAA)_7G$. *Sphaeralcea* banding patterns were much more complex (many more bands) than *Linum*; six dinucleotide primers were used for screening: $(CA)_9A$, $(CA)_9T$, $(GA)_9T$, $(CT)_9T$, $(CA)_9G$, and $(GA)_9A$. For *Penstemon*, we used eight dinucleotide and two trinucleotide primers: $(CA)_9A$, $(CT)_9T$, $(GA)_9A$, $(GA)_9C$, $(GA)_9T$, $(GT)_9A$, $(CAG)_7G$, and $(CTC)_7G$.

PCR reactions consisted of 1.5 μL 10x PCR buffer (Continental Lab Products), 0.9 μL $MgCl_2$ at 50mM (CLP), 2.1 μL 33% glycerine in ddH_2O, 0.375 μL dNTPs at 40 mM each nucleotide (CLP), 1.2 μL primer at 10 μM, 0.15 μL Taq at 1 U/μL (TAQ Red DNA Polymerase, CLP), and 1 μL template DNA, in a 15 μL reaction volume. We included negative control reactions, containing 1 μL ddH_2O in place of the DNA, with each set of reactions. The thermal cycling profile consisted of an initial prewarming step of 3 minutes at 94° C, followed by 33 cycles of 1 minute at 94° C, 1 minute at 55° C, and 1 minute 45 seconds at 72° C, and a final extension step of 2 minutes at 72° C. We used the same profile for all primers. The ISSR product fragments were separated by electrophoresis on agarose gels (1.5% agarose in 0.5x TBE). We loaded 5 μL of each PCR product onto a gel, which ran for three hours at 100V. We included negative control reactions on the same gels with the reaction sets of which they were part. Gels were stained gels with Ethidium Bromide, visualized them with UV light, and photographed.

Numerous factors can create genotyping errors in population genetic data sets. Such errors can be due to chance, to human fallibility, and to technical artifacts, operating at every stage from sampling to data analysis (Bonin et al. 2004). Genotyping errors can result in the presence of fragments in one individual and their absence in another, even when the "true" genotypes of the individuals are identical. Thus, they can be a source of bias in the results. To check the repeatability and consistency of our ISSR protocol, we included duplicate reactions for each individual in the initial primer screens, and selected only those primers for which banding patterns were identical in both reactions for each individual. We also repeated several complete reaction sets, and confirmed that the banding patterns of each individual were identical in the repeated reactions.

Human factors, including lack of experience, subjectivity, and confusion in sample tracking and data entry can be a major source of errors in the process of genotype scoring. Different workers working from the same photographed gels may score genotypes very differently (Bonin et al. 2004). To minimize the bias due to human error in scoring, a single person scored all *Linum* ISSR genotypes. The scoring was done manually, assigning a binary value of '1' for presence of a band in an individual genotype and '0' for absence of a band found in other individuals. To guide the scoring process, we ran gels for each primer on which several individuals from each population were placed side-by-side. Comparing banding patterns across populations and species enabled us to confidently assign bands of similar molecular weight from separate gels to the same ISSR "locus." For *Sphaeralcea* and *Penstemon*, scoring was facilitated by DNAQuantar (http://www.keygene.com), a program which helps determine molecular weight of bands, but which does not eliminate human scoring and interpretation entirely.

Data Analyses

Linum received the most thorough analyses because population sampling was most successful with this forb and the data quality was excellent. The following exposition thus focuses on the *Linum* analyses although the general methodology is applicable to the remaining species groups.

We first used the individual multi-locus genotypes as units of analysis to construct a phenetic cluster diagram representing the genetic similarity of individuals within and between populations (Hollingsworth & Ennos 2004). We generated a pairwise similarity matrix using the Dice coefficient, S=2a/(2a+b+c), as calculated in NTSYSpc (Rohlf 2002). The matrix was transformed to a genetic distance matrix by the transformation D=1-S. We then implemented the Neighbor Joining algorithm in NTSYSpc to produce a phenogram of all individuals. Genetic distance matrices and Neighbor Joining phenograms were constructed separately for the eight single-primer data sets, and for the combined data set of all primers. We used Treeview (Page 1996) to view the phenograms.

Further analyses followed a population-based approach, in which population allele frequencies are calculated and used to estimate F-statistics (Hollingsworth & Ennos 2004). F_{ST} and its analogues measure the partitioning of genetic diversity within and between populations (Hartl & Clark 1997). We used two different approaches to estimate F_{ST}. The first, implemented in TFPGA (Miller 1997), assumes Hardy-Weinberg equilibrium within each population. This assumption is unlikely to be valid, especially in the case of obligately outbreeding *L. perenne*, whose system of mating is characterized by disassortative mating for stylar morph. However, this assumption is necessary in this case to estimate allele frequencies from the observed genotype frequencies at each locus. Under the diploid / dominant marker options in TFPGA, the Taylor expansion-based estimator of Lynch and Milligan (1994) was used to estimate population allele frequencies. These frequencies were then used to calculate such descriptive statistics as estimated observed and expected heterozygosities, and percent polymorphic loci (under a 95% criterion, i.e., the estimated frequency of the "presence" allele is between 2.5% and 97.5%). TFPGA uses the method of Weir and Cockerham (1984) to calculate θ_{ST}, an estimate of F_{ST}. To obtain the estimated variance of the F_{ST} estimates, we jackknifed over all loci (10000 replicates). To obtain 95% confidence intervals for the F_{ST} estimates, we bootstrapped over all loci (10000 replicates). F_{ST} and its variance and confidence interval was estimated separately for each

species. These analyses were performed for each single-primer data set separately and for the combined data set.

We also used TFPGA to perform Fisher's exact tests for population differentiation. These tests use marker frequencies (band presence frequencies), not estimated allele frequencies (Miller 1997). TFPGA incorporates a Markov Chain – Monte Carlo (MCMC) algorithm to approximate the exact probability of the observed marker frequency differences. Fisher's combined probability test is then applied to determine the overall significance of the differences in marker frequencies across all loci (Miller 1997). We used 20 batches of 2000 permutations per batch with 1000 dememorization steps to estimate the p-value. Exact tests for population differentiation were performed on the entire data set; on the populations of each species; and on certain defined contrasts to test hypotheses (e.g. the distinctiveness of the Ibapah population from other populations of *L. lewisii*).

The second method of estimating F_{ST} used a Bayesian approach, implemented in Hickory v1.0 (Holsinger et al. 2002, Holsinger & Lewis 2003, Holsinger & Wallace 2004). This method uses beta distributions to approximate the posterior distribution of θ^B, an F_{ST} analogue under a random-effects model of population sampling (Holsinger & Lewis 2003). The advantage of Bayesian estimation is that one need not assume that the genotypic frequencies within populations conform to Hardy-Weinberg expectations. Indeed, no previous knowledge of the within-population system of mating is required (Holsinger et al. 2002). As recommended, we used the program's default parameters (burnin=50000, sample=250000, thin=50). We estimated F_{ST} for each species separately, again using each of the eight single-primer data sets and the combined data set. In each analysis, we ran both the full model, which estimates the within-population inbreeding coefficient f_{is} along with F_{ST}, and the f-free model, which chooses values of f_{is} at random from its prior distribution while estimating the other parameters (Holsinger & Lewis 2003). It may not be possible to reliably estimate f_{is} from dominant marker data (Holsinger & Wallace 2004); the f-free model is provided to estimate F_{ST} without concern about the possible bias created by unrealistic estimates of f_{is}. To evaluate the differences between models, we sampled log files for each analysis and performed posterior comparisons between the results of different models. We also used posterior comparisons to evaluate differences in F_{ST} between the two species, and thus to test the hypothesis that the native species has greater genetic differentiation between populations than does the seeded species.

RESULTS AND OBSERVATIONS

Linum

Summary Statistics. The number of bands scored per primer varied from 45 to 72. Not all bands for each primer were observed in all populations; usually less than half the total number of bands that were scored for each primer were observed in any given population. In part, this was due to the large number of species-specific bands identified. A total of 164 bands present only in *L. lewisii* and 103 bands present only in *L. perenne* were scored. Of these, 28 were specific to a single *L. lewisii* population, and 22 were specific to a single *L. perenne* population. Thus, 10.6% of all bands scored were found in only a single population. Most of these population-specific bands, moreover, were scored in only one or two individuals. In total, 57 of the 470 bands scored (12.1%) were present in three or fewer individuals in the data set.

While only four species-specific marker bands (i.e., bands present in every individual of one species and in no individuals of the other species) were identified – one each for *L. lewisii* and *L. perenne* in primer CA9A, one for *L. lewisii* in primer GA9C, and one for *L. perenne* in primer GA9T – numerous species-typical bands were found. Species-typical bands are here defined as bands whose frequency in one species exceeds their frequency in the other species by either 0.25 (25% criterion) or 0.50 (50% criterion; Table 4).

Table 4. Summary of DNA ISSR markers in *Linum* sp.

	# bands scored	# bands scored per (full-sized) population (range)	# species-specific bands (*L. lewisii* / *L. perenne*)	# population-specific bands (*L. lewisii* / *L. perenne*)	# individual-specific bands*	# species-typical bands, 25% criterion** (*L. lewisii* / *L. perenne*)	# species-typical bands, 50% criterion** * (*L. lewisii* / *L. perenne*)
CA9A	56	19-31	30 (20/10)	7 (5/2)	4	26 (16/10)	20 (12/8)
CA9G	58	22-33	25 (16/9)	6 (4/2)	7	17 (9/8)	6 (2/4)
CAA7G	64	22-35	36 (25/11)	9 (6/3)	9	28 (16/12)	14 (6/8)
CAA7T	61	18-36	34 (19/15)	4 (2/2)	6	28 (12/16)	14 (7/7)
CT9G	45	10-25	34 (16/18)	10 (2/8)	16	14 (5/9)	10 (3/7)
GA9A	55	12-33	27 (15/12)	4 (3/1)	3	26 (10/16)	14 (6/8)
GA9C	72	28-40	45 (25/20)	5 (2/3)	6	38 (19/19)	25 (14/11)
GA9T	59	14-32	36 (28/8)	5 (4/1)	6	19 (9/10)	9 (4/5)
All primers	470	181-235	267 (164/103)	50 (28/22)	57	196 (96/100)	112 (54/58)

* Individual-specific is here arbitrarily defined as present in 3 or fewer individuals in all populations combined – so some of these are not population-specific or even species-specific.
** Bands for which the frequency in one species exceeds the frequency in the other species by 0.25 or more
*** Bands for which the frequency in one species exceeds the frequency in the other species by 0.50 or more

Modified Fisher's exact tests were also implemented in TFPGA to identify bands whose frequency differed significantly between *L. lewisii* and *L. perenne*. Overall, 356 out of 470 bands (75.7%) were significantly different (at $p<0.05$ level) in frequency between species. For primer CA9A, 47 out of 56 bands (83.9%) showed a significant difference; for primer CA9G, 38 out of 58 bands (65.5%); for primer CAA7G, 46 out of 64 bands (71.9%); for primer CAA7T, 52 out of 61 bands (85.2%); for primer CT9G, 24 out of 45 bands (53.3%); for primer GA9A, 47 out of 55 bands (85.5%); for primer GA9C, 60 out of 72 bands (83.3%); and for primer GA9T, 42 out of 59 bands (71.2%). Despite the significant difference in frequency between the two species, many of these bands are present in such a low frequency that they do not qualify as "species-typical."

Percent polymorphic loci. Polymorphic loci are here defined by a 95% criterion: a locus is polymorphic if its most common allele is present in less than 95% of individuals. For dominant markers, allele frequencies cannot be measured directly. Percent polymorphic loci is here estimated using two different methods. First, polymorphic bands are considered to be those for which the most common state (either presence or absence) occurs in less than 95% of

individuals (Table 5); that is, the frequency of band presence is between 0.05 and 0.95. According to this criterion, 68.7% of all bands scored across all populations are polymorphic. (Most of the rest are low-frequency, with only a few bands in greater than 95% frequency across all populations of both species.) Because many bands were species-specific, less than half of the bands scored are polymorphic within either species. Second, percent polymorphic loci is calculated based on allele frequencies estimated under the assumption of Hardy-Weinberg equilibrium, using the program TFPGA (Table 6). According to this estimate, 54.9% of bands are polymorphic across all individuals, and around 41% are polymorphic within each species. Note that measures of percent polymorphic loci are expected to decrease as the number of sampled populations increases, because each new population potentially adds more population-specific bands to the data set, which will be monomorphic (absent) for all other populations, and most likely will be too low-frequency to qualify as polymorphic for the species as a whole.

Table 5. Percent polymorphic bands (95% criterion), based on observed band frequencies

	all	LL	LP	L1	L2	L3	L4	L5	LA	LB	P4L	P1	P2	P3	P4	P5
CA9A	73.2	50.0	48.2	32.1	21.4	39.3	21.4	23.2	30.4	19.6	8.9	37.5	25.0	33.9	28.6	33.9
CA9G	58.6	50.0	50.0	24.1	24.1	32.8	27.6	43.1	29.3	24.1	19.0	29.3	39.7	36.2	27.6	48.3
CAA7G	70.3	53.1	37.5	42.2	43.8	31.3	34.4	32.8	23.4	20.3	26.6	26.6	25.0	28.1	31.3	26.6
CAA7T	73.8	52.5	45.9	49.2	41.0	29.5	26.2	21.3	27.9	31.1	21.3	42.6	26.2	37.7	36.1	23.0
CT9G	37.8	17.8	22.2	17.8	8.9	15.6	22.2	17.8	24.4	4.4	4.4	17.8	46.7	24.4	22.2	20.0
GA9A	74.5	45.5	60.0	12.7	32.7	29.1	40.0	25.5	27.3	40.0	36.4	43.6	23.6	43.6	49.1	47.3
GA9C	79.2	48.6	46.6	37.5	25.0	20.8	27.8	30.6	26.4	25.0	11.1	30.6	33.3	44.4	22.2	40.3
GA9T	72.9	55.9	40.7	28.8	40.7	25.4	35.6	23.7	35.6	18.6	22.0	18.6	18.6	20.3	32.2	37.3
All primers	68.7	47.7	44.7	31.5	30.4	28.1	29.6	27.7	28.1	23.4	18.9	31.1	29.4	34.0	31.1	34.9

Table 6. Percent polymorphic loci (95% criterion), based on estimated allele frequencies assuming Hardy-Weinberg equilibrium (as calculated by TFPGA)

	all	LL	LP	L1	L2	L3	L4	L5	LA	LB	P4L	P1	P2	P3	P4	P5
CA9A	57.1	42.9	50.0	35.7	25.0	42.9	21.4	21.4	30.4	19.6	32.1	41.1	26.8	35.7	30.4	33.9
CA9G	48.3	37.9	36.2	22.4	24.1	25.9	19.0	32.8	29.3	24.1	19.0	29.3	31.0	22.4	27.6	46.6
CAA7G	51.6	45.3	35.9	40.6	39.1	32.8	31.3	35.9	23.4	20.3	26.6	28.1	21.9	26.6	32.8	23.4
CAA7T	59.0	47.5	41.0	49.2	39.3	29.5	26.2	21.3	27.9	31.1	21.3	41.0	23.0	36.1	34.4	24.6
CT9G	33.3	15.6	24.4	13.3	8.89	13.3	13.3	13.3	24.4	4.44	4.44	20.0	35.6	24.4	24.4	24.4
GA9A	60.0	41.8	50.9	18.2	27.3	30.9	36.4	30.9	27.3	40.0	36.4	43.6	18.2	41.8	47.3	47.3
GA9C	68.1	47.2	47.2	33.3	30.6	27.8	26.4	30.6	26.4	25.0	11.1	43.1	36.1	45.8	29.2	36.1
GA9T	54.2	42.4	37.3	22.0	40.7	27.1	30.5	28.8	35.6	18.6	22.0	16.9	15.3	18.6	30.5	35.6
All primers	54.9	41.1	40.9	30.2	30.2	29.1	26.0	27.4	28.1	23.4	21.7	33.4	26.0	31.9	32.1	34.0

Phenetic relationships. In the neighbor-joining phenograms for each data set, *Linum lewisii* and *Linum perenne* 'Appar' were recovered as wholly separate groups, with very few exceptions. Interestingly, five individuals from *L. perenne* population P4 were consistently placed among *Linum lewisii* for each primer and in the total data set. These individuals were P4.6, 4.7, 4.22, 4.23, and 4.24. Population P4, a roadside seeding in Ephraim Canyon, was collected by workers who had previously collected only *Linum perenne* and who were not trained in distinguishing the two species (without training, species differentiation is difficult after noon by which time the petals have typically fallen from plants). It seems probable that a native

population of *L. lewisii* was present in Ephraim Canyon, adjacent to, and intermingling with, the seeded *L. perenne*. Without recognizing the difference, the collectors sampled these five individuals from two clusters of *L. lewisii* within the sampled area of *L. perenne*. These five are henceforth referred to as a group, "P4L", and were treated as a small population of *L. lewisii* in subsequent analyses (Fig. 1).

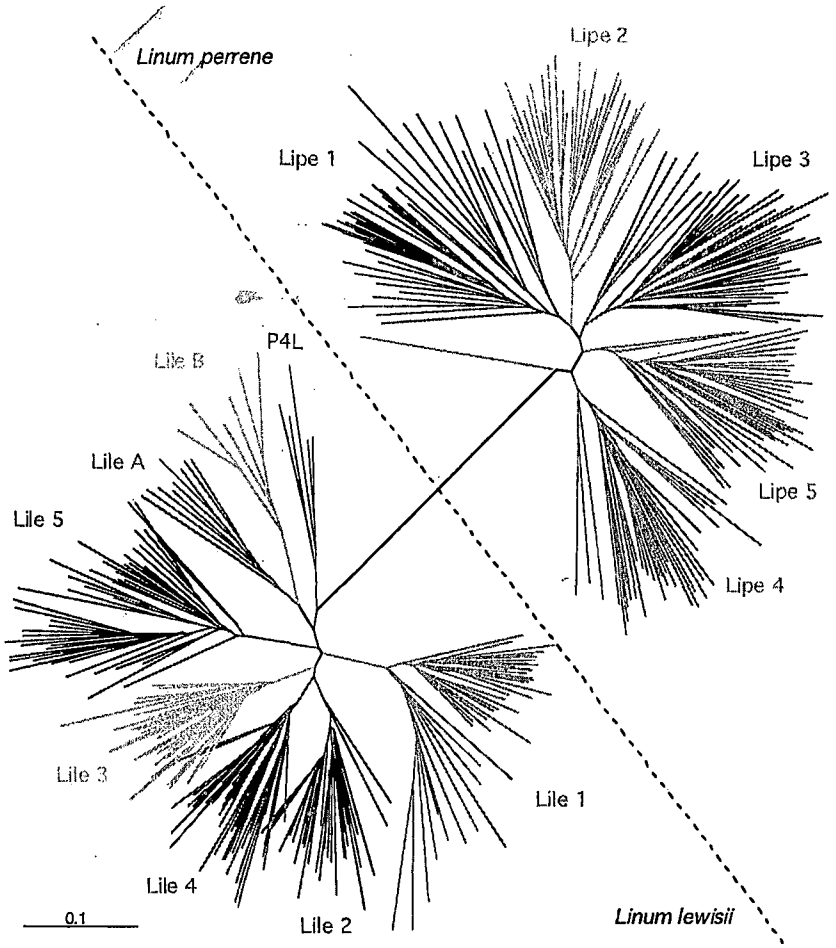

Figure 1. Neighbor joining phenogram of genetic similarity based on complete analysis of 470 loci from eight ISSR primers. Colors are arbitrarily used to denote population locations (Table 1). P4L indicates individuals collected as LIPE 4, but likely representing native LILE growing with LIPE.

Other exceptions to the *L. lewisii* / *L. perenne* separation consisted of a few individuals of both species that, for some primers, were placed basal to all others (or in a very few cases placed within the opposite species). Upon re-examination of the gel photos and scoring sheets, these individuals had been flagged during scoring as possibly subject to contamination; they were consequently removed from all other analyses.

In each of the eight single-primer analyses, no population of either *L. lewisii* or *L. perenne* was recovered as a wholly separate or "monophyletic" group (with the exception, sometimes, of small populations LA, LB, and P4L). The degree of mixing of individuals from different populations varied from primer to primer. For CAA7G, CAA7T, and GA9C, relatively few individuals were placed in the "wrong" population group, but for CA9A, quite a bit of "mixing" occurred. Subjectively, *L. perenne* appears to have more "mixing" of populations than *L. lewisii* in single-primer analyses.

In the total data set consisting of 470 loci, however, populations were recovered as distinct, separate groups, with very few exceptions (Fig. 1) : P5.26 and P3.39 are placed in the basal portion of the P1 cluster, and P5.10, P5.46, and P5.16 are placed in the basal portion of the P3 cluster. In single-primer analyses, the small populations LA, LB, and P4L bounce around a bit, but LA and LB are most often grouped with or near many L5, while P4L often appear with L4. With the total data set, NJ places P4L as sister to LA+LB, and this "small L populations" group sister to L5. The sister relationship between LA and LB may be an artifact of their having been run on the same gel and scored simultaneously.

Exact Tests of Population Differentiation (TFPGA). At the species level, *Linum lewisii* differed significantly (p=0.0000) from *Linum perenne* in marker frequencies, for each of the eight single-primer data sets and for the total data sets. Furthermore, all eight primers, and the total data set, showed significant differences (p=0.0000) in marker frequencies among all sampled populations.

In pairwise comparisons of populations, most were significantly different ($p<0.05$) in marker frequencies across all primers. The small *L. lewisii* populations (LA, LB, P4L), however, did not significantly differ from one another; and P4L did not significantly differ from L4. The same was true of these populations in each of the eight single-primer data sets, due to their small samples sizes (nine, six, and five individuals respectively). In the eight single-primer data sets, all populations of *L. lewisii* always differed significantly from all populations of *L. perenne*. Also, populations within a species usually differed significantly from one another in marker frequencies. Most of the exceptions involved either the small *L. lewisii* populations, which often did not differ significantly from L2, L3, L4, and/or L5; and the *L. perenne* populations, which sometimes did not differ significantly from one another.

Marker frequencies for primer CA9A did not differ significantly (i.e., $p>0.05$) between L4 and L2. Marker frequencies for primer CA9G did not differ significantly between L5 and LA or LB; nor between L3 and P4L. Marker frequencies for primer CAA7G did not differ significantly between L4 and LA or P4L; between L5 and any of the small populations; between P1, P2, and P3; nor between P3 and P5. Marker frequencies for primer CAA7T did not differ significantly between L1 and LB or P4L; between L4 and LA or P4L; between L5 and P4L; nor between P2 and P5. Marker frequencies for primer CT9G did not differ significantly between any of the *L. lewisii* populations, nor between any of the *L. perenne* populations except for P2, which differed significantly from all other populations. This was probably due to the high percentage of low-frequency, population- and individual-specific bands among the 45 bands scored for this primer. Marker frequencies for primer GA9A did not differ significantly between P3 and P5. Marker frequencies for primer GA9C did not differ significantly between P4L and any other *L. lewisii* population; nor between P2 and P3 or P5. Marker frequencies for primer GA9T did not differ significantly between L1 and LB; between L4 and LB or P4L; or between P1, P2, and P3.

Exact tests were also used to compare particular populations of interest. L5, the *L. lewisii* population from Ibapah, was thought to be a potentially distinctive population of this species, on the basis of its unusual habitat type and somewhat different morphology. However, the ISSR data provides no support for this hypothesis. Modified Fisher's exact tests for population differences in marker frequencies, contrasting L5 to L1+L2+L3+L4, were significant (p=0.0000)

for all single-primer data sets except CT9G, and for the total data set. However, the same is true for L1, L2, L3, or L4 contrasted with the other populations. So, these tests provide no support for the hypothesis that the Ibapah population is uniquely distinct from other sampled *L. lewisii*.

The five individuals of population P4 that clustered with *Linum lewisii* in the neighbor-joining phenograms, herein referred to as "small population" P4L, were contrasted with a grouping of the other *L. lewisii* populations, and with a grouping of the other *L. perenne* populations. For each of the eight single-primer data sets, and for the total data set, P4L differed significantly (p=0.0000) from *L. perenne*. However, P4L did not differ significantly from *L. lewisii* for the total data set, or for any single-primer data set except CA9A (p=0.0003) and GA9A (p=0.0001). These tests support the conclusion that these five individuals represent a population of *L. lewisii* intermingled with the roadside seeding of *L. perenne* at site P4, Ephraim Canyon, and show no evidence of interbreeding between the species at this site.

Populations L2 and P2 were collected at the same site, a burned area near Holden, Utah. These populations of *L. lewisii* and *L. perenne* differed from one another as much as did any populations of the two species, and showed no evidence whatsoever of interbreeding between the two species.

Penstemon

Meaningful within and between population inferences in *Penstemon* are limited in this study by the small population sizes encountered for all but one of the reseeded populations of Palmer's penstemon. Instead, we focused on identifying species specific markers that could be used to assess introgression with the co-occuring penstemon species at the three locations where a species other than *P. palmeri* was growing (Table 7).

Table 7. Approximate molecular weights of diagnostic bands unique to each species in sympatric Penstemon populations.

Population/primer	$(CA)_9A$	$(CT)_9T$	$(CTC)_7G$	$(GA)_9T$	$(GA)_9A$	$(CAG)_7G$	$(GA)_9C$	$(GT)_9A$
PEPA A (Twist)						690		
PEEA B (Twist)	356, 436, 558	1390				340,1085	1190	
PECC C (Hwy70)	645	1285			690	340	1190	
PEPA D (Hwy70)		1990				1230	1030	
PECA E (Pole)			790	1125, 425	530, 690, 795			1080
PEPA F (Pole)					1350			1250, 1500, 1835

Sphaeralcea

Whereas hybridization between *Sphaeralcea* species is apparent morphologically, ISSR patterns were complicated to the extent that, although variation could be observed within populations, banding profiles were more or less similar even between distinct species. For example, bands that appear consistently bold in *S. ambigua* and seem diagnostic may be present, but faint, at high frequency in *S. grossularifolia* (Fig. 2a). Likewise, SPGR4, a pure native stand of *S. grossularifolia* is equally heterogeneous to the nearby SPGR3 population that included many individuals showing morphological intermediacy with *S. munroana* (Fig. 2b). At first

glance, it is tempting to score only the intense bands in this group, but this can only be done by ignoring what may be homologous faint bands in other individuals. Polyploidy, which is well documented in this genus, may contribute to the increase in band number and complexity of banding patterns that confounded accurate scoring of variation with confidence. For example, very intense, broad bands may represent two, slightly loci that differ only slightly in length.

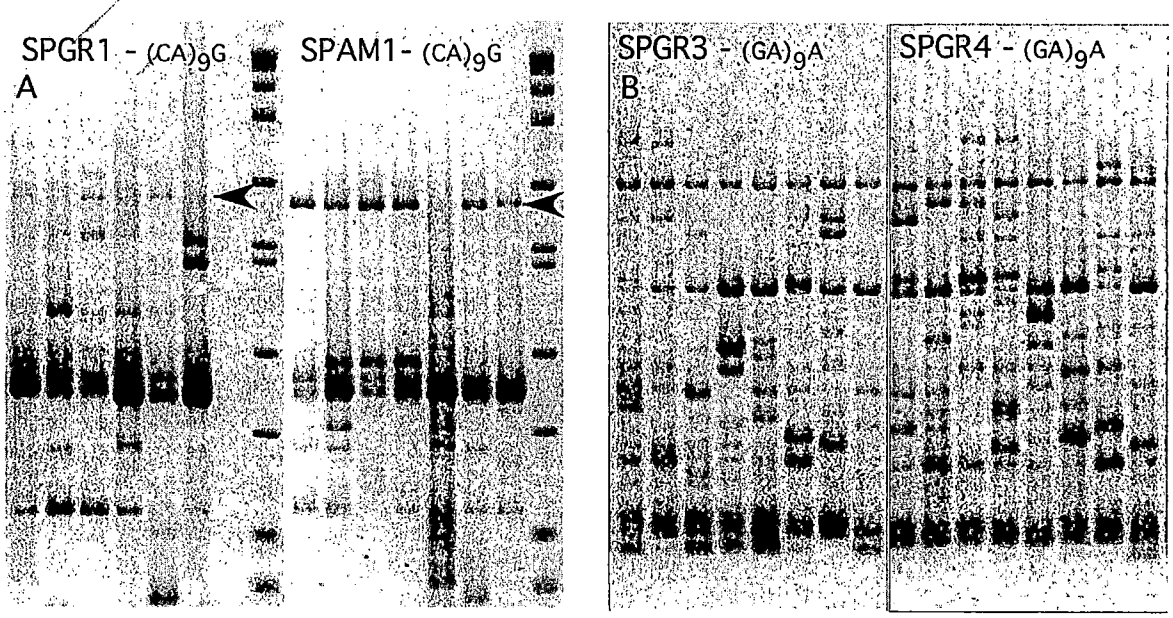

Figure 2. Sample ISSR gel banding patterns for *Sphaeralcea* populations. A. Sympatric *S. grossularifolia* (left) and *S. ambigua* (right). B. *Sphaeralcea grossularifolia* pure stand (right) and parapatric *S. grossularifolia* and *S. munroana* mixed stand, including probable hybrids (left).

CONCLUSIONS

Application of ISSR data.

Despite their usefulness, the dominant nature of ISSR data poses a major challenge for analysis of genetic variation within and among populations (Lynch & Milligan 1994) because homozygotes for presence of a band cannot be distinguished from heterozygotes. Furthermore, the data is treated as bi-allelic, with band absence representing the recessive allele. However, whereas band presence is determined by the presence of two appropriate priming sites separated by a given length of DNA, absence could be created by any number of mutations, including length mutations in the priming sites or intervening regions, point mutations in the tandem repeats or the anchoring base of the priming site, and recombination. In calculating pairwise similarity values, therefore, it is not appropriate to consider shared absences as evidence of similarity (Wolfe & Liston 1998). Nei and Li's (1979) coefficient of similariy, which does not incorporate shared absences, was used in this study. This coefficient is mathematically equivalent to the Dice coefficient (Dice 1945).

The dominant nature of ISSR data also need to be kept in mind when assessing introgression. "Diagnostic" bands present uniquely in a population or species can be evaluated for evidence of introgression, but the absence of the band in an individual belonging to a species

or population that typically shows the band is not evidence of introgression in the reverse direction because band loss can occur via the several mutational routes elaborated above.

Dominant marker data such as ISSRs are often used to generate phenograms, which may be used to infer population structure and gene flow. Intuitively, strong structure in such a phenogram would seem to indicate genetic structure of the populations. However, the relationship between the topology of these phenograms and the actual genetic structuring of the populations may be strongly affected by the number of dominant loci included in the data set (Hollingsworth & Ennos 2004). The clustering of individuals from the same population tends to increase as the number of loci increases. In very large data sets, mutually exclusive clustering of populations in the distance phenogram may be obtained even when F_{ST} is actually rather low. Therefore, inferences about population genetic structure, and corresponding conservation recommendations, should not be based solely on the topology of distance phenograms constructed from dominant marker data (Hollingsworth & Ennos 2004).

Banding complexity and interpretation will impact the inferences drawn from ISSR data. While we used agarose gel electrophoresis to separate fragments, as has been used in many other studies, we suspect that this method underestimates the number of loci and, in the case of Sphaeralcea, results in complex patterns where homology assignment during scoring becomes suspect, even with the aid of software designed for such scoring. The use of florescent labeled primers and fragment separation through either polyacrylamide or packed columns, such as used in, for example, Applied Biosystems automated genetic analyzers, would provide more fine-scale differentiation of fragments, increased confidence in homology assignments, and an increase in number of fragments scored. Future efforts should use such technology.

Species inferences.

ISSR data support the evidence that Appar is a cultivar of *L. perrene*, not *L. lewisii*, and that these species are genetically isolated. While populations of both species show genetic variation, variation is typically higher *in L. lewisii*, which would be predicted for an indigenous species compared to an introduced cultivar; however, the differences in levels of genetic variation are not great. Several bands, while not always fixed within each species, and limited to each species and have the potential to track introgression, although hybridization does not appear to be a concern with these taxa and we observed no evidence of introgression.

In contrast, hybridization is a concern with Palmer's penstemon, although not necessarily between this species and the species we found growing with it in this study. Several species-specific marker bands were identified that could be useful for diagnosing hybrids or the extent of introgression among the taxa studied, if such putative hybrids were found. Presently, we understand little about the isolation mechanisms that may be operating between Palmer's penstemon and the other species surveyed which may prevent or limit hybridization in these species. However, because the seeding material of Palmer's is typically not of the same material native to Utah, consideratin should be given before seeding this species near the native range, and before seeding it near narrowly endemic penstemon species where knowledge of isolating mechanisms is poorly understood.

Few inferences can be made regarding Sphaeralcea based on the genetic surveys conducted here. In general, we noted that S. munroana may be in use in seedings in place of S. grossularifolia, but such inference is based on visual observation rather than through identification of unique genetic signatures among the populations surveyed. Sphaeralcea appears to have high levels of genetic diversity, perhaps maintained via polyploidy, in both seeded and native stands. This diversity complicated our efforts to score visually apparent variation in a reliable way across all individuals, and we recommend use of finer methods of resolving ISSR markers in this species, such as mentioned above, in any future work.

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